

What is claimed is:

1. A genotyping method using a DNA chip on which an optimal probe pair of a wild type-perfect match probe and a mutant type-perfect match probe are immobilized for each mutation site.

2. The genotyping method of claim 1, wherein at least two optimal probe pairs are immobilized for each mutation site of the DNA chip.

3. The genotyping method of claim 2, wherein at least two wild type-perfect match probes are arranged side by side and at least two mutant type-perfect match probes are arranged side by side adjacent to the wild type -perfect match probes for each mutation site of the DNA chip.

4. The genotyping method of claim 1, wherein the optimal probe pair is selected by:

designing a plurality of probe pairs of a wild type -perfect match probe and a mutant type -perfect match probe using an *in silico* method;

immobilizing the plurality of probe pairs on a substrate to manufacture an optimal probe pair screening chip;

hybridizing a standard nucleic acid to the optimal probe pair screening chip;

collecting quantitative hybridization intensity data; and

selecting a probe pair having the largest value calculated using the quantitative hybridization intensity data and the following equation:

$$\{Mean(\ln(r^{wt})) - 2 SD(\ln(r^{wt}))/\sqrt{N^{wt}}\} - \{Mean(\ln(r^{mt})) + 2SD(\ln(r^{mt}))/\sqrt{N^{mt}}\}$$

wherein  $N$  denotes the number of times hybridization of the target nucleic acid has been performed;  $r^{wt}$  is the ratio between the hybridization intensity of a wild type standard nucleic acid to the wild type-perfect match probe and the hybridization intensity of the wild type standard nucleic acid to the mutant type-perfect match probe;  $r^{mt}$  is the ratio between the hybridization intensity of a mutant type standard nucleic acid to the wild type-perfect match probe and the hybridization intensity of the mutant type standard nucleic acid to the mutant type-perfect match probe; and *Means* and *SD* denote the mean value and standard deviation of  $N \ln(r)$  values,

respectively, which are obtained by hybridizing the standard nucleic acid to the DNA chip  $N$  times.

5. The genotyping method of claim 1, comprising:

(a) setting up a genotyping algorithm using data obtained from hybridization of an identified standard nucleic acid to the DNA chip; and

(b) genotyping an unknown target nucleic acid by substituting an input vector that are calculated from hybridization of the target nucleic acid to the DNA chip into the genotyping algorithm.

6. The genotyping method of claim 5, wherein (a) comprises:

(a-1) collecting quantitative hybridization intensity data obtained from hybridization of the identified standard nucleic acid to the DNA chip;

(a-2) calculating the ratio between the hybridization intensity of the standard nucleic acid to the wild type-perfect match probe and the hybridization intensity of the standard nucleic acid to the mutant type-perfect match for every probe pair, selecting the median from among the calculated ratios using Hodge-lehman estimation, and taking the natural logarithm of the median as a ratio component of a vector used to set up the genotyping algorithm; and

(a-3) repeating (a-1) and (a-2) with a plurality of DNA chips to obtain a set of vectors and setting up the genotyping algorithm using the set of vectors.

7. The genotyping method of claim 6, wherein (a-3) comprises calculating logistic regression coefficients for the genotyping algorithm using the set of vectors.

8. The genotyping method of claim 6, wherein (a-2) further comprises multiplying the hybridization intensities of each probe pair, selecting the median from among the products using Hodge-lehman estimation, dividing the natural logarithm of the median by two to obtain an intensity component of the vector used to set up the genotyping algorithm; the genotyping method further comprises plotting a graph with the Y-axis parameterized by the ratio component and the X-axis parameterized by the intensity component before (a-3); and the genotyping algorithm is set up in (a-3) using all of the ratio components if the ratio component of the graph has a independence on the intensity component or using only some ratio components

which are independent of the intensity component if the ratio component of the graph has a dependence on the intensity component.

9. The genotyping method of claim 6, wherein (a-2) further comprises  
5 taking the larger of the hybridization intensities of each probe pair, selecting the median from among the selected larger hybridization intensities using Hodge-lehman estimation, taking the natural logarithm of the median as an intensity component of a vector used to set up the genotyping algorithm; the genotyping method further  
comprises plotting a graph with the Y-axis parameterized by the ratio component and  
10 the X-axis parameterized by the intensity component before (a-3); and the genotyping algorithm is set up in (a-3) using all of the ratio components if the ratio component of the graph has a independence on the intensity component or using only some ratio components which are independent of the intensity component if the ratio component of the graph has a dependence on the intensity component.

15 10. The genotyping method of claim 6, further comprising filtering out quantitative hybridization intensity data obtained from bad spots that have a larger diameter than an effective spot diameter from the quantitative hybridization intensity data collected in step (a-1) before (a-2).

20 11. The genotyping method of claim 5, wherein (b) comprises:  
(b-1) collecting quantitative hybridization data obtained from hybridization of the unknown target nucleic acid to the DNA chip;

(b-2) calculating the ratio between the hybridization intensity of the target  
25 nucleic acid to the wild type-perfect match probe and the hybridization intensity of the target nucleic acid to the mutant type-perfect match for every probe pair, selecting the median from among the calculated ratios using Hodge-lehman estimation, and taking the natural logarithm of the median as an input vector for genotyping; and

30 (b-3) substituting the input vector into the genotyping algorithm to genotype the target nucleic acid.

12. The genotyping method of claim 11, wherein (b-3) comprises calculating the posterior probabilities that the target nucleic acid is wild type or a

mutant type by substituting the input vector into the genotyping algorithm and determining the genotype of the target nucleic acid to be a wild type or a mutant type depending on the greater posterior probability.

5           13.    The genotyping method of claim 11, wherein (b-3) comprises:  
              calculating the posterior probabilities that the target nucleic acid is wild type or  
a mutant type by substituting the input vector into the genotyping algorithm to  
determine the genotype of the target nucleic acid to be a wild type or a mutant type  
depending on the greater posterior probability; and  
10           validating the reliability of the greater posterior probability at a predetermined  
significance level and deferring genotyping of the target nucleic acid if the reliability  
requirement is not satisfied.

15           14.    The genotyping method of claim 11, further comprising filtering out  
quantitative hybridization intensity data obtained from bad spots that have a larger  
diameter than an effective spot diameter from the quantitative hybridization intensity  
data collected in step (b-1) before (b-3).

20           15.    The genotyping method of claim 5, further comprising correcting the  
genotyped results from step (b) based on cross-hybridization data of the probe pair  
for each mutation site.

25           16.    A DNA chip used for a genotyping method, comprising an optimal  
probe pair of a wild type-perfect match probe and a mutant type-perfect match probe  
which are immobilized for each mutation site.